Check List the journal of biodiversity data

NOTES ON GEOGRAPHIC DISTRIBUTION

Check List 17 (3): 753–758
https://doi.org/10.15560/17.3.753

PENSOFT

A new occurrence of *Mucor inaequisporus* Dade (Mucorales, Mucoromycota) from soil of the Atlantic Forest in the Brazilian Northeast

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Abstract

Mucor inaequisporus Dade (Mucorales, Mucoromycota) was isolated for the first time from soil in an area of Atlantic Forest in the state of Alagoas, Brazil. It is distinguished from other species by simultaneously producing erect, undulating and curved sporangiophores, as well as mostly pyriform, oblong, conic, ellipsoid, and obovoid columellae. The sporangiospores vary in size and shape, with some irregular in shape. Aspects of the morphology and distribution of this species are commented on.

Keywords

Alagoas state, Mucoromyceta, Mucoraceae, taxonomy

Academic editor: Renan Barbosa | Received 23 October 2020 | Accepted 8 April 2021 | Published 11 May 2021

Citation: Freitas LWS, Lima CLF, Souza CAF, Cunha GCL, Leitão JDA, Silva SBG, Cruz MO, Oliveira RJV, Santiago ALCMA (2021) A new occurrence of *Mucor inaequisporus* Dade (Mucorales, Mucoromycota) from soil of the Atlantic Forest in the Brazilian Northeast. Check List 17 (3): 753–758. https://doi.org/10.15560/17.3.753

Introduction

The Atlantic Forest is a domain with high diversity and endemism that may encompass 1–8% of the world's total biodiversity (da Silva and Casteleti 2003). However, only 28% of its natural vegetation cover remain in a fragmented state because of extensive human activity (Marques et al. 2007; Rezende et al. 2018). This alarming loss has given the Atlantic Forest the status of hotspot and has put areas of this domain on the priority list for the conservation of global biodiversity (Galindo-Leal and Câmara 2003). To date, only 2,702 species of fungi have been reported in the

Brazilian Atlantic Forest from which 66 species belong to the order Mucorales (Flora do Brasil 2020).

Mucorales, the largest order of Mucoromycota, encompass about 300 species (http://www.indexfungorum.org) of which nearly 90 belong to the genus *Mucor* Fresen. (Wijayawardene et al. 2020). *Mucor* taxa are characterized by forming simple or branched sporangiophores, which emerge directly from the substrate, and globose or subglobose, non-apophysate sporangia (Benny 2014). Some taxa produce rhizoids (e.g. *M. luteus* Linnem. ex Wrzosek and *M. irregularis* Stchigel, Cano, Guarro & Ed. Álvarez), and stolons are not formed. So far, 31 species of *Mucor* have been isolated from the Brazilian Atlantic Forest, mainly in Pernambuco and São Paulo states (Flora do Brasil 2020).

According to Schipper (1978) and Santiago et al. (2013), *M. inaequisporus* Dade is mainly characterized by forming a yellow colony with unbranched or sympodially branched sporangiophores, with columellae and sporangiospores highly variable in form and size. This species has been recorded from Ghana (Dade 1937), Indonesia (Boedijn 1958), Japan (Naganishi and Hirahara 1966), Malaysia (Williams and Liu 1976), Iran (Zangeneh et al. 2007), Brazil (Santiago et al. 2013), and South Korea (Lee and Jung 2020).

Here, we present a description and illustration of *M. inaequisporus* isolated from soil samples from an area of Atlantic Forest in Alagoas state, Brazil. This is the second report of this species in South America, but the first record from the Brazilian Northeast.

Methods

Soil samples were collected in February 2019 in the biological Reserve of Pedra Talhada, which is located in Alagoas state (Fig.1), Brazil. For isolation, 5 mg of soil were added to Petri dishes containing wheat germ agar medium (Benny 2008) amended with chloramphenicol (80 mg/L). The dishes were left on the laboratory bench at room temperature (28 ± 2 °C) for 7 days in alternate periods of light and dark. Fragments of mycelium were transferred separately to malt extract agar (MEA) (Benny 2008), with added chloramphenicol (80 mg/L). Fragments of selected fertile areas of colonies were removed from the plates for examination of fungal structures. These were placed, together with a drop of KOH (3%) or lactophenol blue, on microscope slides and observed under a light microscope (Carl Zeiss Axioscope 40). The specimen was identified by comparing the macroscopic and microscopic characteristics as described by Dade (1937), Schipper (1978), Santiago et al. (2013), and Lee and Jung (2020). Sixty-five measurements were made for each fungal structure. The strain was deposited in the Culture Collection Micoteca URM

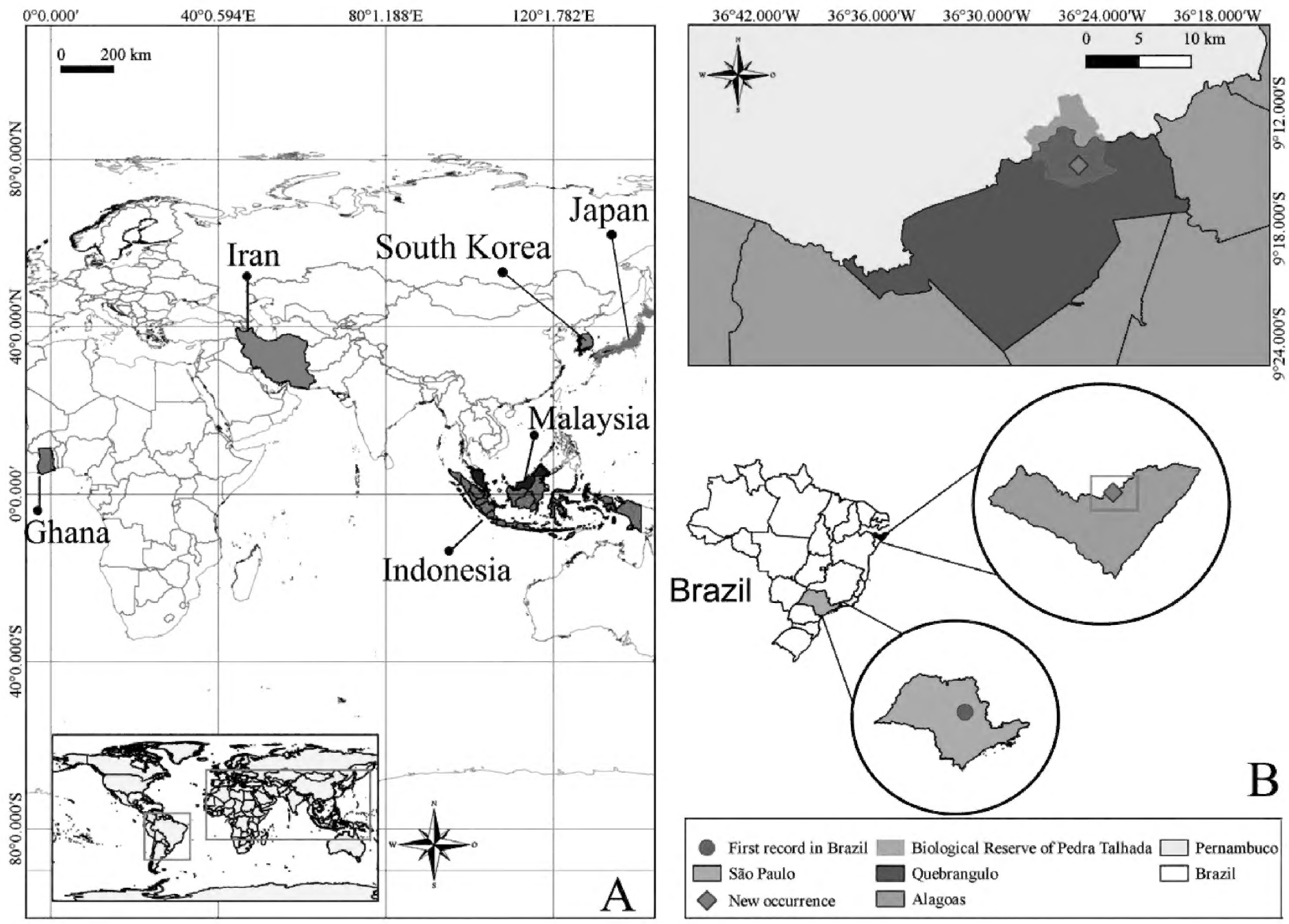

Figure 1. A. Geographical distribution of *Mucor inaequisporus* in other countries. **B.** First record of *M. inaequisporus* in Brazil and the new occurrence (URM 8105).

of the Universidade Federal de Pernambuco, Recife, Brazil. Information regarding the distribution of *M. inaequisporus* was retrieved from published manuscripts, the Global Biodiversity Information Facility GBIF (https://www.gbif.org), GenBank (https://www.ncbi.nlm.nih.gov/genbank), and from plutoF platform (https://plutof.ut.ee).

The fungal biomass was obtained for genomic DNA extraction as described by Oliveira et al. (2016). The primer pairs ITS1/ITS4 (White et al. 1990) were used to amplify the ITS region rDNA. The polymerase chain reaction was conducted as described by Oliveira et al. (2014). The newly obtained sequence was deposited in GenBank (MW826106). Phylogenetic reconstructions were obtained by analyzing sequence data of the ITS rDNA. The sequence obtained was aligned with some other related fungal sequences from GenBank using MEGA v. 5.05 (Tamura et al. 2007). Prior to phylogenetic analysis, the optimal model of nucleotide substitution (HKY+G) was estimated using TOPALi v. 2.5 (Milne et al. 2004). The maximum likelihood analysis (with support estimated by a bootstrap analysis with 1000 replicates) was performed using PhyML (Guindon and Gascuel 2003), in TOPALi v. 2.5. The phylogenetic tree was visualized using Treeview (Page 1996) and edited using Inkscape v. 1.1 (https://inkscape.org/en/).

Results

Phylogenetic Analysis. In the phylogenetic tree (Fig. 2) the sequence of URM 8105 was grouped in a clade together with other sequences of *M. inaequisporus,* including the holotype (CBS 255.36/JN206177).

***Mucor inaequisporus* Dade**, Trans. Br. mycol. Soc. 21 (1–2): 25 (Dade 1937)
Figure 3

Material examined. BRAZIL • Alagoas, Quebrangulo, Biological Reserve of Pedra Talhada; 09°14′42.9″S, 036° 25′14.4″W; 739 m a.s.l.; 1 February 2018; Leslie W. Freitas leg.; habitat: soil; URM 8105, 1 specimen.

Identification. Colonies golden yellow, reverse yellow, growing rapidly on MEA (9 cm diameter) after 4 days at 25 °C. Sporangiophores yellow, erect, undulating, curved and constricted next to the sporangia, with yellow droplets, simple or with long or short sympodial branches, with encrusted wall, 12–45 µm in diameter. Sporangia yellow, globose and subglobose, with a vitreous aspect, 60–170 µm in diameter, wall echinulate, deliquescent in mature sporangia and persistent in young sporangia. Columellae yellow, highly variable in shape, pyriform, some with a constriction, conic, oblong,

Figure 2. Phylogenetic tree of *Mucor inaequisporus* and related species constructed using ITS rDNA sequences. *Entomortierella parvispora* was used as outgroup. Sequences are labeled with their database accession numbers. Support values were obtained from maximum likelihood analysis. The sequence obtained in this study is annotated in bold. Only support values of at least 80% are shown.

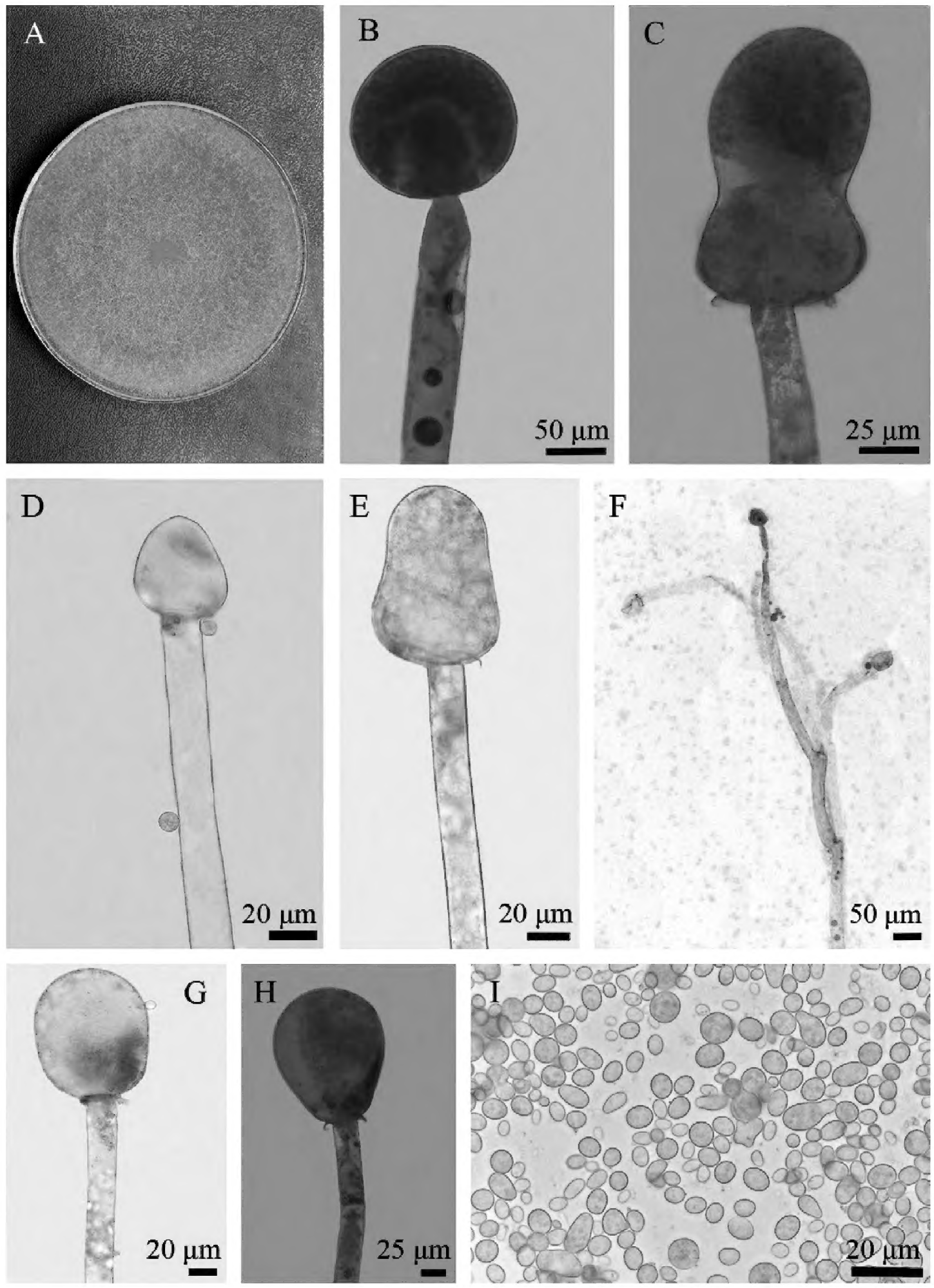

Figure 3. *Mucor inaequisporus* (URM 8105). **A.** Surface of colony on MEA at 25 °C. **B.** Unbranched sporangiophore with sporangium. **C–E.** Unbranched sporangiophore and columella. **F.** Sympodially branched sporangiophore and columellae. **G, H.** Unbranched sporangiophore and columella. **I.** Sporangiospores. Structures on B, C, F and H were stained with lactophenol blue.

ellipsoid, obovoid, subglobose with yellow granules, with a prominent collar, smooth-walled, 33.5–110 (–130) × 40–90 (–110) µm. Sporangiospores yellowish green, with granular contents, variable in shape and size, ellipsoid, 5–13 (21.5) × 3–11 (–16) µm, globose and subglobose, 7–18 µm in diameter, some irregular, 12–30 × 5–12 (–16) µm, smooth-walled. Chlamydospores and zygosporangia not observed.

Discussion

To the best of our knowledge, *Mucor inaequisporus* has been reported once from soil; Zangeneh et al. (2007) isolated it from the rhizosphere of root knot nematode host plants in Iran. Dade (1937) found this species on fruits of Hog Plum, *Spondias mombin* L., in Ghana; Williams and Liu (1976) isolated it from *Theobroma cacao* L., in Malaysia, and Boedijn (1960) isolated it from *Artocarpus glaucus* Blume, *Flacourtia inermis* Roxb., *Musa paradisiaca* L., *Diospyros kaki* L., and *Bouea macrophylla* Buwga in Indonesia. Santiago et al. (2013) isolated it from mature fruits of *Syzygium cumini* L., in São Paulo state, Brazil. Lee and Jung (2020) found this species causing persimmon (*D. kaki*) fruit rot in South Korea.

The descriptions of this species by the above-mentioned authors are similar, although Dade (1937), Schipper (1978), and Santiago et al. (2013) observed a few variations in relation to the size of columellae. Schipper

(1978) described collumellae with 83–75 µm, smaller than the ones observed by us and Santiago et al. (2013) (up to 120 × 140 µm). All these authors noted that the columellae are variable in shape, although predominantly pyriform. We also observed some constricted columellae in our specimen. Santiago et al. (2013) reported sporangiophores with randomly distributed irregular swellings, a characteristic not observed in URM 8105 and not described by Dade (1937) and Schipper (1978). Yet, Lee and Jung (2020) reported morphological characteristics of *M. inaequisporus* similar to those given by Santiago et al. (2013).

We report the first occurrence of *M. inaequisporus* in Northeastern Brazil, specifically from soil of an Atlantic Forest area, contributing to the knowledge of the geographical distribution of Mucoralean fungi.

Acknowledgements

We thank the National Council of Scientific and Technological Development (CNPq) for the scholarships provided to Leslie W.S. Freitas, Joana D.A. Leitão, Catarina L.F. Lima, and for the research grant provided to André C.M. de A. Santiago. This work was financed by the projects "Diversity of Mucoromycotina in the different ecosystems of the Atlantic Rainforest of Pernambuco" (FACEPE-First Projects Program PPP/FACEPE/CNPq-APQ-0842-2.12/14) and "Biology of conservation of fungi in areas of Atlantic Forest of northeastern Brazil" (CNPq/ICMBio 421241/2017-9).

Authors' Contributions

CLFL, CAFS collected the material and formatted the plate; JDAL, SBGS, and GCLC performed the specified methodology; MOC prepared the map; RJVO performed the molecular analyzes; LWSF and ALCMAS wrote the text and identified the specimen.

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